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The Determination of Structure and Reactivity at the Surfaces of Materials Used in Biology: Needs and Requirements for Electron and Ion Spectroscopy for Surface Analysis

by

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The determination of structure and reactivity at the surfaces of materials used in biology: needs and requirements for electron and ion spectroscopy for surface analysis

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Abstract

We discuss here the status and challenges of the use of surface chemical analysis based on electron and ion spectrometry for "biomaterials", that class of materials and their applications where the primary surface contact of a polymer, metal, alloy, ceramic, or semiconductor, etc. is with a biological environment. Subsequent papers in this special issue highlight contrasting views on the relevance of surface science to this problem, the emergence of scanning probe microscopy and applications in areas of interest to surface scientists. The challenges of structure determination and the relationship with reactivity in these environments are outlined. Some examples are given describing areas for future growth of electron and ion spectroscopy. These are highlighted by problems in the analysis of reactive materials; where the purpose of the material is not to be "inert" to the biological milieu. Surface chemical problems of general import include (bio)-adhesion and (bio)-corrosion; thus, there are direct parallels with other areas of substantial previous work in surface science.

Keywords: Biodegradable polymer; Fluoropolymer; Metals and alloys; Reactivity; SIMS; Surface structure; XPSN

1. Introduction. The surface chemistry of biomaterials. Are there challenges and opportunities for surface scientists?

What defines a biomaterial? What special role does surface structure and reactivity play? Very generally, the purpose of a biomaterial is to replace a part or a function of the body. In order to achieve that purpose successfully, biomaterials should possess adequate mechanical, physical and chemical properties; they should continue to perform their functions for the duration of the application and not induce unacceptable changes in the surrounding biological milieu, be it fluid, tissue, cells, etc. Therefore, interfacial interactions are critical, and a biomaterial must exhibit a specific surface chemical behavior in addition to the required bulk properties.

Even though the range of materials used in biological applications is broad, from polymers to metals and alloys, to ceramics, glasses and even semiconductors, and also considering the wide range of applications, in order to become a successful biomaterial, all materials should be able to

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perform a function in a biological environment without adversely affecting it. There are several important issues that need to be addressed in the study of the interaction between the material and the biological system. In this context, research on surface properties of biomaterials, especially concerning tissue reactions over both short and long term, is very valuable, not only for improving systems based on metals and polymers already in use, but also for expanding into new biomaterials.

This paper will attempt to set the basis for this special issue of the *Journal of Electron Spectroscopy and Related Phenomena* dedicated to the Surface Chemistry of Biomaterials. A number of questions and concerns have driven a recent rise in interest among surface physicists, chemists and engineers in this field of endeavor. The measurements of surface structure, composition and reactivity are, in the view of the present authors, as directly relevant to serious problems and challenges in this field of materials and surface science, as they are in other fields. In fact, it is our view that there is much in common with this field and others important in the recent history of the growth of surface science. This can be based on the linkage of the growth of surface science, in general, to advances in measurements such as electron spectroscopy and the importance of surfaces in problems in catalysis and semiconductor solid state and surface structure.

An attempt will be made to present the argument that there are many important reasons why electron and ion spectroscopy and microscopy should be used to analyze biomaterials, and that the information is *directly relevant* to the performance of the material in the biological milieu. This view is countered by a companion article in this issue by Dr. E.A. Vogler: readers may recognize similarities to the debates over the "pressure gap" in the field of catalytic surface chemistry with respect to the interpretation of mechanisms in ultra-high vacuum versus applications of catalytic materials in normal use. The rest of the issue consists of reviews of relevant surface chemical studies in classes of materials of importance in biomaterial application, spanning metals, alloys, ceramics and polymers.

We shall focus on five remaining points of discussion in the present paper. Firstly, we shall describe the current state of a general model of biomaterials surface interactions. Secondly, we shall present an overview of the state of electron and ion (mass) spectroscopic analysis of surfaces for problems in biomaterials. Thirdly, we shall present a case study which illustrates how surface chemical composition and structure, as determined by ex situ surface analysis, lead to a direct effect on the structure of adsorbed proteins, which in turn lead to a direct effect on cellular adhesion and growth. The fourth point discussed is challenges and reviews of issues in the reactivity of polymer and metal surfaces. Making progress in this area is a major challenge for the future of surface science measurements in biomaterials. Finally, we conclude with our views of the prospects for surface analysis in the field in the coming years.

2. Problems and models in the surface science of biomaterials

There is a tendency, noted in the Foreword to this issue, to think of the field of biomaterials as being dominated by the development of materials for prosthetics for human application. A major historical driving force in the field was the development of materials for the artificial heart. Yet, as many recent reviews have noted [1,2], the applications of biomaterials where surfaces are important are diverse, ranging from the use of catheters in the short term, to invasive and non-invasive biosensors, to sutures and bandages for wound healing, materials for drug delivery, contact lenses, dental materials, along with the more complex materials used for orthopedic replacements (e.g. hips, knees, joints) and cardiovascular applications (valves, veins). The issues for defining successful biomaterial–surface interactions depend on the application; however, common ground has been developed within the model described below and is schematized in Gristina's review [2]. That common ground has evolved the field to recognize similarities in designing the surfaces of materials used for exposure to marine environments, and other areas in environmental application where contact with biological systems is important.

Obviously, biodegradation (whether to be avoided or encouraged) also requires a view of surface chemistry. Similarly, biocorrosion may occur in any of these environments.

In order to achieve a better understanding of biomaterial–cell surface interactions, it is important that each interface be considered. In evaluating a material for a biomedical application, the following approach has been suggested [3]:

(ii) The surface of the material under consideration should be fully characterized in terms of its chemistry (elemental/molecular composition), physical morphology, and structure.

(ii) The interactions of macromolecules in the biological system with the characterized surface should be studied.

(iii) The cellular response to the material should be evaluated by performing in vitro and in vivo experiments.

The key to achieving success is an interdisciplinary approach to the research by combining expertise in the fields of physics, chemistry and biology.

Two roads to understanding are commonly taken in materials science. One way involves an understanding through analysis of materials used in real applications. In the field of biomaterials this has great value. Often, however, choices about what material is used in a particular biological application have not considered the state of the surface which is utilized. A second, more fundamental approach, is to construct a model system which can simulate the environment of application, and learn as much as possible about the fundamental steps which control success. This latter approach has attraction for most physical scientists, of course, but is hampered by the lack of agreement on the fundamental issues in constructing the model, and the complexity of disciplines (materials science and engineering, cell and molecular biology, biomacromolecular structure and function, biophysics, etc.) which must be integrated to even understand the jargon of discourse in the field.

However, progress has been made in the latter arena. A hierarchical approach would involve design and analysis of the material ex situ (e.g. outside a (natural) biological environment), applications of the materials in a so called fundamental model in vitro (outside the living body and in an artificial, generally biological environment), and then finally test studies in vivo (in the living body of a plant or animal). Understanding the issues in modern in vitro testing remains a significant barrier for most physical scientists to surmount, yet there may still not be relevant to real in vivo applications.

Some features of a model which can test and understand materials surface chemistry in vitro have been made. The interaction of the surface of a biomaterial with the biological environment in which it is inserted is a highly dynamic process beginning at the time and point of implantation. Glycoproteinaceous conditioning films coat the biomaterial almost immediately [4], and provides receptor sites for tissue adhesion. These conditioning films act as molecular bridges between the cells and biomaterial surfaces, the strength of binding being a function of the proteinaceous film and the underlying surface.

This model of biomaterial/cell surface interaction has been summarized by Gristina [2]. A schematic diagram of that model is presented in the figure in the accompanying paper by Vogler [5]. It is suggested that the success of an implant can be conceptualized as a race for the available surface in which biological macromolecules, bacteria, and cells compete for the surface. The success or failure of the implant will be determined by the conditioning macromolecules. If the race is won by these cells, a stable integration would be achieved making the surface less suitable for bacterial colonization, which would lead to infection.

3. Methods for surface characterization of biomaterials

An important initial stage in the evaluation of biomedical materials is the surface characterization of the material. Substantially different chemical properties at the surfaces compared to those observed in the bulk can be induced by a variety of physical–chemical mechanisms. A few examples are the presence of surface contamination, a specific molecular orientation at the surface, and surface

specific chemical reactions. Because the surface properties cannot always be predicted from observation of the bulk properties, surface characterization studies have an essential value in the research, development and production of biomaterials.

Surface characterization can also be used in the identification of bulk impurities (especially those which bloom or segregate to the surface) and for general quality control. The need to verify that the surface in question is a clean, pure material, or that modification reactions have been successful, is vital to a successful characterization/implantation analysis.

Surface analytical techniques are also necessary to fully understand what was referred to as the second stage: the effects of the surface of the material on the adsorbed conditioning film. Several papers in the recent literature, have demonstrated a systematic approach to the characterization of a surface aiming to better understand the role of chemical functional groups on surface–proteins–cells interactions.

A number of selected surface analysis methods have been applied to biomaterials. Modern methods of surface analysis differ from classical approaches. Classical methods of surface analysis include measurements such as adsorption isotherms, surface areas, pore size distribution, surface roughness, thickness (ellipsometry) and surface topography via microscopy. Modern spectroscopic methods, in contrast, provide information such as elemental composition, oxidation states, and functional groups, quantitative analysis and distribution of species, both lateral and in the bulk [6].

The four techniques with the widest range of applicability are X-ray photoelectron spectroscopy (XPS or ESCA), secondary ion mass spectrometry (SIMS), Auger electron spectroscopy (AES), and ion scattering spectroscopy (ISS). XPS and SIMS, in particular, have shown their potential for biomedical investigations [7].

SIMS is the technique in which the developments are currently most rapid [8]. Although SIMS has been extensively used to provide surface molecular information and to depth profile materials, the application of the technique to study biomaterials began only in the early 1980s. The advancement experienced in recent years has been remarkable.

XPS has not only been used to characterize biomaterial surfaces [9,10], but also in the characterization of material–tissue interfaces [11] and for detecting biomolecules adsorbed at surfaces [12].

In addition to the surface spectroscopic methods mentioned above, X-ray fluorescence spectroscopy (EDX) and Fourier-transformed infrared spectroscopy (FTIR) are often used. These methods, however, are not surface sensitive in the same sense as the surface spectroscopies. FTIR, frequently used in studies of the interaction between biomolecules and surfaces, has proven to be a very valuable source of information [13–15].

Recently, scanning tunneling microscopy (STM) and surface force microscopy (SFM or AFM) [16,17] have made it possible to characterize surface structures at subnanometer and even atomic resolution. Several research groups are currently applying the superior resolving power of these nanoprobes to the study of biomaterials [18,19].

4. Does surface analysis contribute to the understanding of surface related adhesion mechanisms from cell biology? A case study

In an attempt to address the question posed by the above heading, and in answer to the questions raised by Vogler [5], we offer the following analysis of ongoing work from our laboratories [20–31]. A series of novel two step surface chemical modifications of fluoropolymers have been described in this work, and the materials were characterized by XPS, infrared, contact angle measurements of surface thermodynamics, SIMS, fluorescence spectroscopy and electron microscopy. One particular application of these materials has focused on designed surfaces for cell adhesion and potential wound healing and tissue engineering applications. In particular, much of the work has focused on potential neural prosthetics.

In our view, the answer to the question posed above is affirmative. The following studies provide direct and clear evidence that the surface chemistry described by ex situ and UHV analysis is relevant to biological processes such as protein adsorption and cell adhesion in the relevant (in this case) in vitro experiments.

The work originated when work in our laboratory yielded a means to incorporate reactive hydroxyl functionality along the fluoropolymer chains of various fluoropolymeric meshes and membrane materials. Much work was accomplished using poly(tetrafluoroethylene) (PTFE), expanded PTFE (ePTFE) and poly(hexafluoropropylene-*co*-tetrafluoroethylene) (so called FEP). Initial work established that reactive hydroxyls could be introduced using a radio frequency inductively coupled plasma discharge composed of hydrogen gas and liquid vapors, most work using water or methanol as a source of hydroxyls in the plasma [20,21]. The first publications established the conditions for synthesizing the new materials surfaces and that this hydroxyl modification (e.g. PTFE-OH, ePTFE-OH, FEP-OH), unlike any other wet chemical or plasma modification previously described, preserved the morphology of the surface, which was especially important for ePTFE membranes [21]. The second paper demonstrated refunctionalization of the ePTFE-OH with amino propyl siloxane (APS) condensation reactions to produce an aminated surface (e.g. FEP-APS or FEP-NH_2) in monolayer coverages [22]. A third paper in this series demonstrated micro lithographic modification using masking technologies [23] in the plasma, followed by the appropriate chemical reactions, under the working assumption that the unmodified fluoropolymer would not react. The resulting micro lithographic patterns were visualized with results from imaging time of flight SIMS. SIMS images were constructed from scanning ion intensities due to unmodified fluoropolymer (yielding a map of the unreacted regions) and APS.

These approaches yielded a series of materials all based on surface modifications of transparent fluoropolymer membranes, without destruction of the membrane morphology, and allowing patterning for visualization of competitive surface reactions side by side.

At about the same time, we began collaborating on the applications of this material to various biosensor and biomaterial applications. In particular, we examined the effects of the electrical properties of a piezoelectric fluoropolymer, poly(vinylidene fluoride) (PVDF) on the growth of neural cells

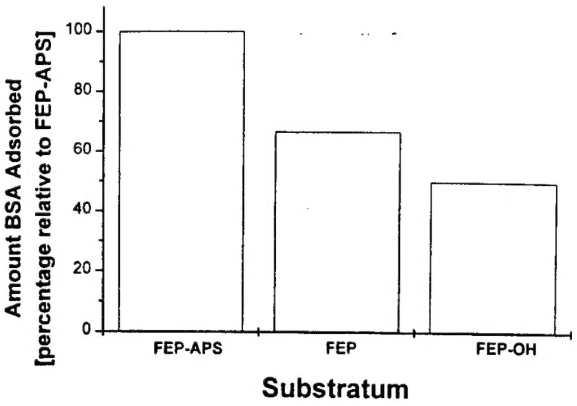

Fig. 1. Comparison of levels of adsorbed bovine serum albumin on fluoropolymer surfaces: percentages relative to adsorbed amounts on amine modified fluoropolymer (cf. Ref. [27]). Quantified using radiolabeled bovine serum albumin.

(both unmodified surfaces [24] and patterned, modified surfaces [25]), showing that the effects of surface modification with patterned PVDF-APS could enhance neurite outgrowth (a step in the differentiation and growth of neural cells). We also showed that ePTFE-APS could be linked to antibodies which could serve in an optical biosensor configuration [26] based on fluorescence detection; this sensor based on fluoropolymer substrata showed unusual stability compared with the same chemistry prepared on silica or quartz glass. We attributed the stability of the protein to the unusual surface chemical environment of the fluoropolymer (more hydrophobic) which would not denature the protein, and deactivate it, as would attachment to fused silica or quartz substrata.

In an attempt to focus more specifically on the unusual surface environment of the fluoropolymer substrata, and the means of using patterns to visualize direct comparisons of surface chemistries, two studies of controlled minimal media through protein adsorption and cell growth were undertaken. In this work, the general idea was to minimize the "interference" of the proteins added to the in vitro cell biological growth conditions, which can adsorb. These media proteins are added as nutrients for normal (or controlled) cell growth. A few cell lines can grow and be studied in "serum free" media, i.e. without added proteins, which potentially minimizes the complexities of interpreting substratum chemistry effects. However,

few studies of surface effects of materials have accounted for these conditions.

In any case, the experimental design of continuing work examined the effects of controlled preadsorption of specific proteins followed by their effects on cell growth for the various modified fluoropolymer substrata [27,28]. FEP, FEP-OH and FEP-APS were studied first with adsorption of bovine serum albumin (BSA) only; BSA is known to passivate against cell growth on fluoropolymers. On the unmodified FEP, this is what occurred, i.e. no cells attached. However, neural cells attached to and grew quickly on BSA adsorbed on FEP-OH and FEP-APS. Different amounts of BSA were clearly adsorbed on the three materials (Fig. 1). Further, the BSA was less easily displaced from the FEP-APS; it was more tightly bound, and adsorbed even at very low temperatures. All this evidence, plus fluorescence spectroscopic studies of the BSA configuration [28] showed that the BSA adsorbed on the FEP-APS was in a different configuration, and that this configuration stimulated cell growth. Other preadsorbed proteins, such as fibronectin, did not show this effect. Thus, by adsorbing this protein onto the FEP-APS surface we could change its structure, and its function, from a passivation agent to an activation agent!

Clearly, the underlying surface chemistry, as characterized by the surface analysis, had a direct influence on the cell attachment and growth.

The current stage of our research [29–31] has taken the next step in materials design. By incorporating specific peptide sequences known to trigger specific cellular events (e.g. attachment, growth, differentiation) using covalent chemistry with the FEP-OH [29], we showed that these surface bound peptides can also stimulate cell attachment [30], although they are at concentrations much lower than the condensed monolayer amine groups in the FEP-APS. The surface attachment was verified with XPS and FTIR; covalently bound peptides were sequenced using TOF SIMS. Patterned surfaces of surface bound peptides [31] directed cell growth along lines and patterns similar to the results from the FEP-APS.

Clearly, these series of results are certain evidence that the surface chemistry of a polymer can be defined by XPS, SIMS and other surface science methods, ex situ, and have a direct impact on cellular events. Further, these studies demonstrate that the surface chemistry can be used to direct and control such events.

5. Issues in surface reactivity

It is clear that the next step in contributing to the understanding of materials in biological environments is for surface science to examine the role of surface reactivity. Many materials have been designed as biomaterials for active applications; the materials have defined capabilities to interact in a controlled way with the biological system. What is very important to understand is the principle that all materials interact with biological systems; there are no "inert" biomaterials. However, the critical use of reactive materials is just now being explored. Two areas of application where surface science techniques can have a clear and definitive role are explored below.

Degradable polymers are considered to be very important materials because of their numerous environmental implications and biomedical applications. Degradable materials are much needed in the manufacturing of disposable/recyclable products such as containers and sanitary products, which otherwise would create environmental build-up. Biomedical applications, however, make use of the susceptibility of these materials to biodegradation, i.e. their degradation in the human body makes them suitable for products with temporary functions such as resorbable sutures, devices used in reconstructive surgery, controlled-release drug delivery systems, and others [32].

Biodegradable sutures, after being useful in wound healing processes, are resorbed by the body, thus eliminating the need to remove the sutures and reducing the possibility of infection. Biodegradable materials for controlled-release drug delivery systems can release drugs in a predetermined way and can deliver some drugs, such as peptide or protein-based drugs, that cannot be administered by other methods.

At present the research on bioabsorbable and soluble polymers is focused on optimizing the use

of degradable polymers and the development of alternative degradable materials [33]. The role of surface degradation processes versus bulk erosion is one of the most relevant issues. The degradation can proceed either in the bulk or at the surface of the material. Fig. 2 shows a particular model of what could control either mechanism in a biological environment. Depending on the pH conditions external and internal to a degradable polymer, either mechanism can dominate [34]. Bulk degradation is useful for applications such as degradable plastics for packaging. Surface degradation, however, is desirable in applications such as drug delivery systems. In order to maximize control over release, it is desirable for a system to degrade only from its surface. In systems with surface erosion, the drug release rate is proportional to the rate of erosion of the polymer. This eliminates the possibility of dose "dumping" (uncontrolled drug release) and facilitates device design.

Over the recent years surface analytical techniques including XPS, SIMS, FTIR, fluorescence spectroscopy and most recently STM, have proven to be powerful tools for studying the surface of polymeric biomaterials. Nevertheless, only in very few instances have these techniques been used to study surface degradation. The development of such a methodology that would allow evaluation of surface degradation by spectroscopic techniques is a goal in our laboratory.

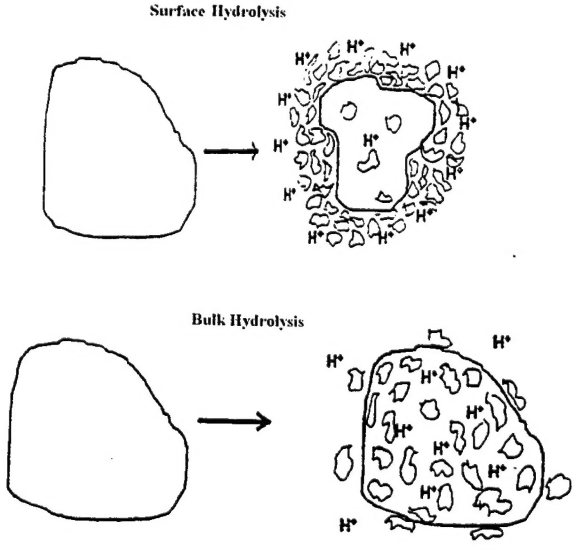

Fig. 2. Model of surface versus bulk etching conditions for a biodegradable polymer which undergoes hydrolysis via acid catalyzed mechanisms.

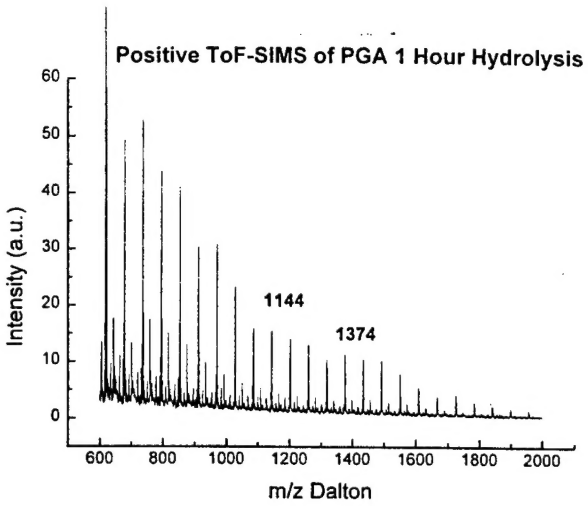

Fig. 3. Typical TOF-SIMS spectrum of the results of a surface hydrolysis experiment on poly(glycolic acid). A distribution of secondary ions related to oligomeric reaction products from 600 Da to 200 Da is shown.

The polyesters are a family of degradable polymers that has received considerable attention. These polymers degrade via a hydrolysis process. The hydrolysis of poly(glycolic acid) (PGA) has been studied in recent work [35–37]. Differential scanning calorimetry (DSC) was used to determine the hydrolysis effects on the bulk of the samples and XPS was used to follow the reaction as it affected the surface [35]. While DSC showed that the samples became more crystalline when subjected to hydrolysis, XPS was not capable of detecting any changes at the sample surface. Moreover, XPS was also unable to detect hydrolysis at the surface of two other polyesters, poly(ϵ-caprolactone) and poly(lactic acid) [35]. The question arising from these results was, could any other surface analytical technique detect the degradation of the polyesters? Static SIMS results demonstrated such promise [36,37].

It has been known for some time that static SIMS is more sensitive to some types of surface degradation on polymers, because of the specificity of the information and the low detection limits.

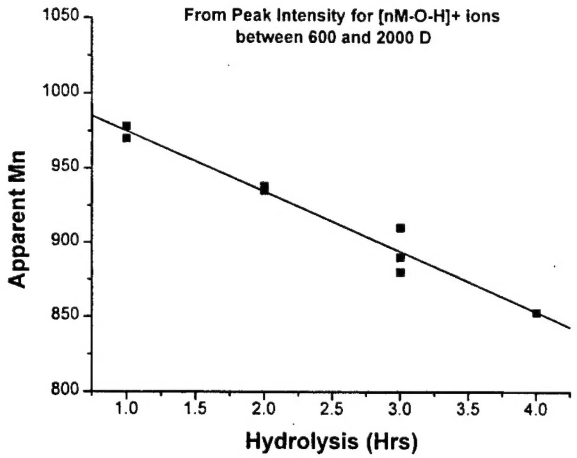

Fig. 4. Plot of apparent number average molecular weight (M_n) of oligomeric poly(glycolic acid) (PGA) hydrolysis products versus in vitro hydrolysis time (hours) for PGA plates etched in biological buffer.

Gardella et al. [38] studied the hydrolysis of poly-(tert-butylmethacrylate) (PtBMA). Static SIMS was used in this work to analyze a system which could not be followed by routine XPS analyses. The results obtained demonstrated the capabilities of SIMS to detect the extent of a mild reaction on the surface of polymers. This work suggested SIMS has the most promising surface technique for the analysis of degradation in polymer surfaces. Hence, SIMS was evaluated as a method for studying the surface degradation of PGA [36]. By using the ability to detect higher mass fragment ions due to oligomeric structures (e.g. Fig. 3) [39], Gatica was able to demonstrate that oligomeric species were present on the surface of plates of PGA after exposure to in vitro hydrolysis conditions. Using a novel set of assumptions that the hydrolysis produced a distribution of oligomeric species which would decrease in average molecular weight when hydrolysis occurred at the surface of PGA, the number and weight averaged molecular weights of the species detected were ratioed and plotted as a function of hydrolysis time (Fig. 4); these data were also subjected to kinetics models to compare autocatalytic and noncatalytic hydrolysis models.

Clearly, there is a role for modern surface science techniques to play in understanding the mechanisms of the surface degradation reactions of biodegradable polymers.

Besides these classes of materials with defined degradation properties, similar issues are also important for metal biomaterials. Metals have widespread applications as biomaterials for orthopedics and dental implants. Among the metals in use one can include stainless steel, cobalt–chrome–molybdenum alloys, titanium, and titanium alloys. Stainless steel is being substituted by other metals because of limitations such as higher corrosion rates and release of nickel ions which can induce undesirable reactions. Presently, the most advanced commercial materials for orthopedic and dental implants are titanium and titanium alloys. These metals have excellent corrosion resistance and very good biocompatibility properties [32].

Even when there has been considerable research carried out on metals as biomaterials, there are issues that require further investigation: interaction of the metal with biological pathways, and non-electrochemical degradative mechanisms including protein–metal interactions [40].

The surface chemistry, oxidation, and dissolution kinetics of titanium were studied by Healy and Ducheyne [41] in an attempt to elucidate the mechanisms of passive dissolution in physiological environments. AES and XPS were used to identify changes in oxide stoichiometry, adsorbed surface species, and oxide thickness as a function of exposure to a model physiological solution [42–44]. Upon immersion, the chemistry of the surface changed: the presence of OH groups increased and P was detected at the surface. The XPS data suggested that a lipoprotein and/or glycolipid film was adsorbed onto specimens exposed to the serum [44].

Amino acids were adsorbed from aqueous solutions onto flat surfaces in order to provide a better understanding of the interaction between organic macromolecules or cells, and the TiO_2 surface upon adsorption [12]. The adsorption as a function of pH was monitored quantitatively by XPS. The results showed that, in acidic aqueous solutions, the amino acids tend to adsorb onto TiO_2 surfaces by their carboxyl groups replacing a basic hydroxyl. Adsorption at basic pH was not observed.

When the implants are inserted in the human body, there is an inflammatory response in which

hydrogen peroxide is released. The outcome of this interaction between the implant material and the hydrogen peroxide is very important. Free radicals, potent agents for cellular deterioration, are formed at this stage and the fate of the implant may well depend on its ability to sustain or stop the free radical formation. For the titanium–tissue interface a model has been proposed where the surface is covered by a TiOOH matrix with apparent (supposedly) desirable ion- and protein-exchange properties [45]. Using XPS and Auger depth profiling, Gatica [36] showed that this surface could be produced on titanium metal coupons, in vitro, by exposure to hydrogen peroxide, but not with $TiAl_6V_4$ alloy, which has been shown to have a complex TiO_2/Al_2O_3 surface. Further, it was shown that this surface was reactive, inducing detectable degradation of model proteins; neither unreacted surface was degradative under the same conditions. Thus, it was proposed that the inflammatory response to a metal implant could drastically change the structure and reactivity of this supposedly "inert" and certainly biocompatible class of metal implant materials.

Surface analysis of in vitro models has clearly elucidated much about the surface chemistry of titanium; more data are reviewed in the papers by Lausmaa [9,46].

6. Conclusions and future directions

The future of surface science in biomaterials is, as described in the Introduction, quite promising. However, much work needs to be done among surface scientists to develop a level of sophistication about the problems in structure and reactivity similar to that developed in other areas, such as catalysis and semiconductor materials.

It is clear that many challenges in structure determination are extant in the field of biomaterials; they are driven mainly by the breadth of materials classes in use in biological environments, and the breadth of "biological" systems in which materials are used. Advances in scanning probe microscopies are especially relevant given the complexity of adsorbed species which need to be detected. Electron and ion based spectroscopies can clearly make determinations which are valuable in understanding the mechanisms and operations of biomaterials. From complex compositional determinations of practical working surfaces to the fundamentals of adsorption of biological molecules at well defined surfaces, atomic, molecular and electronic structures are important in determining surface properties.

An especially detailed set of challenges for the future of the field involves the definition of reactivity at biomaterials surfaces. As outlined in this paper, biomaterial reactivity is not simply the result of complexity of surface interactions of "passive" or "inert" materials. Many "reactive" materials with designed applications are important, although there is little fundamental knowledge of surface reactivity, changes in structure and composition, and their relationship to bulk properties. Using both microscopy and spectroscopy to determine the mechanisms and kinetics of surface reactions, will be a challenge further complicated by the effects of protein adsorption and tissue integration.

Finally, these authors would encourage a detailed development of expertise among surface scientists in the language of molecular and cellular biology. The skills to communicate across the boundaries of other fields of application are necessary to understand and design relevant experiments and studies. Surface scientists are encouraged to fine-tune their well developed skills in crossing intellectual boundaries to compete in this exciting field.

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